

COM701, A FIRST-IN-CLASS IMMUNE CHECKPOINT INHIBITOR, TARGETS A NOVEL
TUMOR PROMOTING PATHWAY MEDIATED BY PVRIG LIGATION TO PVRL2

by
Matthew S Neil

A thesis submitted to Johns Hopkins University in conformity with the requirements for the
degree of Master of Science

Baltimore, Maryland
July 2018

Abstract

Cancer is a leading public health crisis facing adults in the United States, with estimations that greater than 1.7 million new cancer cases and over 600,000 cancer-related deaths will occur in 2018 (1). While the cost of cancer has continued to increase, therapeutic advances in the treatment of cancer have grown more specific (2). This specificity has led to decreased toxicity, improved patient tolerance, and increased efficacy (3). Radiation and chemotherapy are no longer the double-edged swords of the past; fractionated dosage, brachytherapy, and 3-D conformational treatments have made radiotherapy more precise and less toxic (4). Meanwhile, chemotherapy has undergone its own transformation. The discovery of new types of chemotherapy has expanded physicians' repertoire for dealing with cancer. Along with surgery, these treatment modalities have proven to be curative for some malignancies (3).

Although cancer-associated mortality rates have dropped by 26% over the past two and a half decades (1), a large amount of work remains to be accomplished. One avenue that has been explored is through the usage of targeted therapy. Increased understanding of molecular biology has presented the opportunity to tailor treatments to patients. Oncologists can now use monoclonal antibodies (mAb) to individualize therapies to each malignancy (5). Using mAbs, oncogenic pathways in the tumor microenvironment can be targeted.

Immunotherapy represents another treatment modality for oncologists. Immunotherapy leverages the strength of the immune system to destroy malignant cells. The immune system is naturally involved in the defense against cancer. Subsequently, for cancer to progress, it must not only overcome inherent oncolytic cellular processes, but also evade immune surveillance. The

goal of immunotherapy is to boost the effectiveness of the immune system in order eliminate cancer cells.

The purpose of immune checkpoints is to prohibit an immune response. The aim of checkpoint blockade is to promote immune activation by decreasing suppressive signaling (6). PD-1 and CTLA-4 were the first two checkpoints targeted. They both have been able to improve outcomes in diseases states that were previously hopeless (7).

Researchers are interested in identifying new checkpoint molecules that could have similar positive effects either individually or in combination. PVRIG is one of these molecules. This protein is expressed on the cell surface of immune cells. It is structurally similar to TIGIT and plays a similar role in regulating immune cell activation (8). PVRIG, TIGIT, and PD-1 inhibit T-cell activation along the same axis.

This paper will review the structure and function of PVRIG. It will focus on its novel role in the immune system and its potential as an immunotherapy. Recent preclinical data will be analyzed, and next steps will be addressed.

Advisor: Drew Pardoll MD, PhD

Acknowledgements

I want to thank the members of the Compugen group in the Pardoll lab. Thank you for all your help throughout the years. Special thanks to Dr. Sudipto Ganguly for your guidance and mentorship. Dr. Drew Pardoll is a paragon of humility, kindness and wisdom. I've told many people before that your successes could not have gone to a more deserving person. I've never said this before, but I aspire to be like you one day.

Thank you to the Graduate Program in Immunology as well as the 4th floor of CRB I. Thanks to you, I've thoroughly enjoyed every moment of my time at Hopkins. Our faculty are not only knowledgeable, but approachable as well. Our students aren't just productive, but kind and self-sacrificing. Class of 2014: Ali, Brian, Chirag, Ian, Sho, and Wendy you guys felt like family from day 1. 4th floor of CRB, I appreciate the resources you've all provided when I failed to plan accordingly, and technical knowledge when I failed to understand anything (everything?). Thank you to Angela and Dr. Joel Pomerantz for your hard work, understanding, and guidance for completing my degree.

Family and friends, you know the whole story, and I appreciate your kind words and encouragement every step of the way. Dad, Heidi, Miles, Mom, Shannon, and Tiffanie: I could never put into words how grateful I am for your presence in my life.

Table of Contents

Abstract	ii
Acknowledgements	iv
Table of Contents	v
Background	vi
Cancer.....	1
Early Non-Surgical Treatments.....	2
Radiation.....	2
Chemotherapy.....	3
Immune Checkpoint Blockade	5
Programmed Death 1 (PD-1).....	6
T cell Immunoglobulin and ITIM domain (TIGIT).....	8
PVRIG	9
Results.....	11
The Role of PVRIG in Cancer Immunology.....	11
Pre-Clinical Studies of Novel PVRIG Blocking Antibody COM701	12
Discussion	14
Figures.....	16

Table of Figures

Figure 1: PVRIG is parallel, non-redundant immune checkpoint in the DNAM/TIGIT axis	16
Figure 2: Binding of PVRIG/TIGIT with increasing concentration of anti-PVR/PVRL2	17
Figure 3: Tumor growth of WT or TIGIT KO mice with control or anti-mPVRIG.....	18
Figure 4: PVRIG binding on CD8 T cells and NK cells on TILs from lung cancer and renal cancer patients.....	19
Figure 5: Expression of PD-1, PVRIG, and /or TIGIT on exhausted T cells from various cancers	20
Figure 6: TGI and survival plots of mice treated with control, anti-mPD-L1, and/or anti-mPVRIG (Ab 407).....	21
Figure 7: Rationale for combination therapy with anti-PD-1, anti-PVRIG, and anti-TIGIT.	22
Figure 8: COM701 effects on CD4 T cell proliferation and CD8 T cell killing using healthy donor cells.....	23
Figure 9: IFN γ production following combination blockade with COM701, anti-PD-1, and/or anti-TIGIT.....	24
Figure 10: IFN γ secretion from TILs extracted from various cancers following blockade of Pembrolizumab (anti-PD-1) or COM701 and/or COM902 (anti-TIGIT).....	25
Figure 11: Lung CD3 TIL secretion of pro-inflammatory cytokines following blockade with COM701 and/or Pembrolizumab.....	26
Figure 12: PVRL2 expression on PD-L1 ⁺ or PD-L1 ⁻ tumors	27
Figure 13: PVRL2 expression on various normal and malignant tissues.	27

Background

Cancer

Cancer has been a part of human existence for centuries. The first recorded instance of cancer being described in a medical context was in 1600 BC. The Edwin Smith Papyrus describes breast cancer tumors and notes that "there is no treatment (9)." For the next 30 centuries, knowledge of the human body continued to grow; however, understanding of cancer did not (10). Improvements and development of new tools, such as microscopy and x-rays respectively, provided the foundation for determining epidemiology of malignancies in humans (11,12). The nomenclature used to describe malignancies has evolved over time.

All cancerous growths were initially characterized as tumors. These tumors were then classified primarily based on their tissue of origin. This verbiage is still used today for example: breast cancer and colon cancer. Along with the development of the modern microscope, came the ability to define cancers by their cell of origin, for example, melanoma and leukemia. Progress in molecular biology combined with the invention of new imaging devices led to more precise nomenclature. Acute myeloid leukemia with translocation or inversion in chromosome 3 is one of over twenty AML diagnoses (13). AML diagnoses are primarily differentiated by genetic mutation and/or cell maturation. Breast cancer clinicians have even more specific diagnoses: T1N2M0, Stage 2, -/-/-, IIIB (14). Each aspect of the breast cancer diagnosis reflects the treatment plan for the patient.

Early Non-Surgical Treatments

Radiation

Radiation was discovered in the late 19th century and was recognized as medically relevant shortly afterwards. Thanks to the efforts of Marie Curie and her husband, radium became a staple of cancer treatment by the dawn of the 20th century. Although radium is no longer used in the clinic, the field of radiotherapy has transformed into a critical component of cancer care. Along with surgery and chemotherapy, radiation therapy is one of the three major treatment modalities for cancer. Radiation therapy is a part of the treatment plan for around half of all tumors (15). It is also an extremely efficient method of treatment. While radiotherapy is believed to account for approximately 40% of curative treatment, it only costs about 5% of all cancer care (15).

The goal of radiotherapy is to prevent cancer cells from dividing. The ionizing radiation that is used damages DNA and prevents cells from proceeding through the cell cycle. Radiation affects both cancerous and healthy cells; however, healthy cells are more capable of repairing their DNA, meaning malignant cells are relatively sensitive to radiation (16). Radiotherapy is divided into two categories based on whether the radiation source is external or internal to the patient. Internal radiotherapy, known as brachytherapy, can be temporary, permanent, or systemic. External beam therapy, teletherapy, is more common than brachytherapy and accounts for nearly 90% of radiotherapy.

Traditional radiation treatments were limited by toxicity. Clinicians were forced to target a large area around the tumor leading to undesired side effects and the inability to treat certain organs (16). The modern era of radiotherapy began with two major developments in the 1980s.

Firstly, the standardization of the linear quadratic model for determining radiation effects provided radiologists with a more accurate way of estimating fractionation doses (17). Secondly, widespread availability of novel imaging devices, including computed tomography scanners and magnetic resonance imagers, enabled oncologists to view malignancies in vivo and target tumors directly (4).

Image guided radiation therapy is now incorporated into every form of radiation treatment. CT scans and MRI images were quintessential in evolving radiotherapy from 2-D to 3-D (16). Intensity modulated radiation therapy divides beams in a manner such that malignant cells receive the primary dose of radiation, while surrounding blood vessels and other healthy tissues are spared (18). Stereotactic radio-surgery relies on precise targeting of an immobile target to deliver a one-time lethal dose of radiation. This technique is now used in place of brain surgery. By focusing external beams onto a single area, patients can benefit from the killing of tumor cells without the cost of invasive surgery (19). Increased understanding of radiobiology, along with improvements in the precision of radiotherapy, make radiation an integral part of modern medicines combinatorial approach to cancer treatment (16).

Chemotherapy

Chemotherapy is a German term that was coined in the early 1900s by Paul Ehrlich. It defines a category of chemical agents that are used to treat diseases. While this definition encompasses almost all treatments other than radiation or surgery, chemotherapy began to refer to cancer treatments in the 1950's (20). It is now specified to be a class of drugs which uses inhibition of DNA replication to kill cancer cells (21).

The United States started many programs during World War II which led to the development of multiple chemotherapy agents that are still used today (3). Gilman and Goodman, who had gained experience working with mustard gas exposed soldiers, were tasked with finding therapeutic effects for these compounds. They discovered that nitrogen mustard caused tumor regression in a mouse model of lymphoma (22). Their findings led to a successful clinical trial (23). Their research led to the development of new class of alkylating chemotherapy drugs including cyclophosphamide (9).

Based on research showing that folate deficiency mimics the effects of nitrogen mustard, Farber and Kille used the antifolate drug currently known as methotrexate, to achieve remission in children with leukemia (24). Around the same time, Elion and Hitchings developed two drugs that affect alanine metabolism. 6-thioquanine and 6-mercaptopurine whose usage in a variety of disease settings would earn them the Nobel prize (25). After noticing increased uracil uptake by hepatic cancer cells, the Heidelberg lab designed a fluorinated pyrimidine which selectively inhibited the growth of malignant liver cells. He was the first person to develop a drug aimed at solid cancers, and the first to create a targeted therapy (26).

Targeted therapy has yielded a silver bullet for the treatment of chronic myeloid leukemia. Chronic myeloid leukemia was known to be associated with the Philadelphia chromosome. The Philadelphia chromosome is a fusion between chromosomes 9 and 22, specifically the genes *BCR* and *ABL proto-oncogene 1* (27). The fusion product created from these genes was later found to be a tyrosine kinase. Since this protein was found in 95% of CML patients and was believed to be required for transformation, it represented an ideal target for therapy (26). Druker et al. was able to develop a compound which displayed sub-uM specificity to the fusion protein and was only cross-reactive to platelet derived growth factor receptor. The

compound exhibited tumor growth inhibition (TGI) in vivo and approximately 95% decrease in BCR-ABL1⁺ colonies in vitro (26). Since the introduction of this drug into the clinic, chronic myeloid leukemia five-year survival rates have doubled from 30% to over 60% (28).

Immune Checkpoint Blockade

Early immunotherapies, such as interleukin-2 (IL-2) and adjuvant focused vaccines, were moderately successful. Although these treatments were effective in clearing the initial tumors, they were not capable of clearing metastatic cancer or preventing metastases (29). Identification of the activation and targeting processes of cytotoxic T lymphocytes (CTLs) by advances in immunology provided another pathway for immunologists to control cancer.

Researchers, seeking more durable responses, began to focus on CD8 T cells (30). Immunologists primarily focused on targeting neoantigens which are only found in the tumor microenvironment (31). Initial results indicated that the immune system reacted to antigens specific to the patient's malignancy. In the early 90s, melanoma researchers demonstrated that tumor specific CD8 T cells were not recognizing neoantigens. In fact, melanocyte specific antigens were the dominant epitopes for the CD8 response to melanoma (31).

The knowledge and theories of the autoimmunity community became critical for cancer immunologists due to the discovery that tumor specific CTLs target self-antigens. The field of autoimmunity hypothesizes that the presence of autoantigens is a normal but latent aspect of a normal immune system (31). A dysfunctional autoimmune response to host antigens breaks self-tolerance. Self-tolerance is a quintessential component of the immune system (32). Regulating

lymphocyte activation is one way that self-tolerance prevents autoimmunity. Lymphocyte activation depends not only on abundance of activation signals, but also on insufficiency of inhibitory signals. It is this balance that prevents autoimmune responses and permits activation against foreign antigens (33). According to the autoimmune theory, increased density of self-antigens during an active immune response can expand a previously minuscule population of T cells sufficiently to initiate an autoimmune response (31).

Since a population of cells exist that can target malignant cells and expand in response to increasing tumor burden, the intention changes from initiating an immune response, to increasing the initial response. The initial response is against a self-antigen, a conventionally inhibited reaction; therefore, the goal is to induce an autoimmune response by suppressing its inhibition (31).

Programmed Death 1 (PD-1)

PD-1 was initially discovered in 1992 while searching for genes related to apoptosis (34). This group noted an autoimmune phenotype in PD-1 knockout mice. Further studies revealed that autoreactive PD-1 deficient mice suffered from splenomegaly and graft versus host disease. Honjo et al. interpreted their findings to indicate that PD-1 was a negative regulator of the immune system. Around the same time, Lieping Chen's group discovered a ligand to an unknown receptor and labeled it B7 homolog 1 (B7-H1) (35). Pardoll and colleagues discovered another B7 family ligand believed to be dendritic cell (DC) restricted, which they named B7-DC (36). Shortly afterwards, Gordon Freeman and colleagues identified that both B7 ligands bound to PD-1 (37). They are currently known as PD-L1 and PD-L2 respectively (38).

PD-1 is a monomeric cell surface protein which contains one variable immunoglobulin (vIG)-like domain in the extracellular portion. Although the extracellular IgE domain shares 25% similarity to cytotoxic T-lymphocyte antigen 4 (CTLA-4), the intracellular domain of PD-1 contains different signaling sequences (40). PD-1 exhibits its downstream effects through an immunoreceptor tyrosine-based inhibitory motif (ITIM) and an immunoreceptor tyrosine-based switch motif (ITSM). Upon ligation of the T cell receptor (TCR) or the B cell receptor (BCR), these motifs recruit phosphatases resulting in inhibition of receptor and local costimulatory signals (38).

PD-1 expression is increased on tumor infiltrating lymphocytes (TILs) who exhibit decreased killing ability. Tumor cells often overexpress PD-L1 as well (41). Engagement of PD-L1 on tumor cells by PD-1 on TILs provides survival signals for the malignancy, while simultaneously inhibiting T cell activation. For these reasons, blockade of PD-1 signaling was viewed as an ideal target (42).

The results of PD-1 blockade in the initial clinical trials were so positive that larger multi-center trials began immediately (43). These treatments have increased overall survival rates and progression-free survival in patients with advanced metastatic malignancies. Importantly, some patients with ongoing disease were able to stabilize and survive after discontinuing treatment (44). The success of PD-1 blockade as monotherapy has led to an explosion of combination therapy clinical trials.

T cell Immunoglobulin and ITIM domain (TIGIT)

Three groups identified different aspects of the same protein in 2009. Grogan and colleagues named the protein TIGIT and recognized that poliovirus receptor (PVR) was one of its ligands (45). They also identified poliovirus receptor-like ligand 2 (PVRL2) as a second, low affinity ligand for TIGIT. In their paper, they describe TIGIT as indirectly suppressing T-cell activation through DCs. The Mandelboim group showed that TIGIT directly inhibits natural killer (NK) cell killing (46). Marco Colonna and colleagues revealed high expression of TIGIT on follicular CD4 T cells and PVR on follicular DCs. Taken together, they hypothesized a role for TIGIT in germinal center interactions (47).

TIGIT is a member of the nectin/nectin-like binding receptor family, which includes DNAX accessory molecule 1 (DNAM-1) and poliovirus receptor-like immunoglobulin domain containing (PVRIG). It contains one extracellular immunoglobulin domain as well as a single intracellular ITIM. TIGIT functions on the cell surface as a homodimer and has high basal expression on regulatory T cells and NK cells (48). Activation of CD4 and CD8 T cells leads to its upregulation, promoting its inhibitory functions (49). TIGIT inhibition in *cis* is mediated by interrupting DNAM-1 dimerization, thereby preventing DNAM-1 from sending activation signals (50). Additionally, TIGIT competes with DNAM-1 for the ligand PVR. TIGIT engagement on activated T cells suppresses TCR and CD28 signaling pathways by downregulating proteins that make up the TCR complex (48). On NK cells, TIGIT's ITT motif mediates inhibition through the phosphatase SHIP1 which downregulates PI3K and MAPK signaling (51).

TIGIT is an attractive target for cancer immunologists because its blockade can increase activation and inhibit suppression (52). TIGIT blockade can preclude suppression by preventing the induction of immunosuppressive DCs and subsequent skewing of T cells from cytotoxic to inflammatory. In the tumor microenvironment, TIGIT blockade can directly promote malignant cell killing by both NK cells and CD8 T cells; additionally, it can stifle immunosuppressive signals from regulatory T cells (53). These factors have led to a surge in publications and clinical trials using TIGIT alone and in combination with PD-1 to target malignancies (54).

PVRIG

PVRIG is a newly discovered nectin/nectin-like receptor. Ben Koop and colleagues discovered the gene analyzing the *PILR* locus. It was named due to sharing variable immunoglobulin domain with poliovirus receptor-like genes (55). Another 10 years passed before researchers would determine its role. In 2016, Zhu et al. characterized the structure and binding partner for PVRIG (56).

PVRIG is a monomeric cell surface protein with one vIg domain and an ITIM-like motif on the intracellular domain. NK cells and CD8 T cells express the protein at rest, while stimulation induces expression on CD4 T cells and upregulates expression on NK and CD8 T cells (57). Zhu and colleagues demonstrated that PVRL2 is the primary ligand mediating PVRIG interactions between T cells and DCs or tumor cells. Results from their competitive binding assay show that PVRIG has a higher affinity for PVRL2 than TIGIT or DNAM-1. Blockade of PVRIG increases T cell proliferation and cytokine production by CD4 T cells and NK cells. These effects were abrogated by anti-DNAM-1 and increased by anti-TIGIT (56,58). Their

findings suggest that co-blockade of PVRIG synergistically enhance T cell function in a manner mediated by DNAM-1.

Results

PVRIG is a novel immunomodulatory receptor. At the time of this writing, a search of Pubmed returns only four results. As indicated by the paucity of publications, there are still many questions to be answered regarding its role in both healthy and diseased states. In collaboration with Compugen, the Pardoll lab has investigated the influence of PVRIG in cancer models, with the goal of translating pre-clinical findings into improved patient outcomes.

PVRIG and TIGIT co-blockade represents a multi-faceted approach to promote TIL activation through DNAM-1. High affinity bindings of PVRIG to PVRL2 and TIGIT to PVR, prevent DNAM-1 ligation. (Figure 1). Blocking these two inhibitory receptors not only increases available ligands for DNAM-1, it also abrogates their intracellular suppressive signaling.

The Role of PVRIG in Cancer Immunology

Using flow cytometry as a readout, Pardoll et al. demonstrate that there is no promiscuous binding of PVRIG to PVR or TIGIT to PVRL2 on Expi293 cells (Figure 2A). The same effect is shown by interferon-gamma (IFN γ) production from donor T cells. Blockade of both PVRIG and TIGIT pathways enhances IFN γ production to 2000 pg/mL; however, blockade of either pathway alone results in approximately 1500 pg/mL increase of IFN γ .

Functionally, co-blockade leads to a significant reduction in tumor growth (Figure 3). TGI is highest in TIGIT KO mice treated with anti-mPVRIG. PVRIG is known to be expressed on healthy NK cells (56); however, Pardoll and colleagues are the first to validate expression on

cancer patient samples (Figure 4). The data from figure 5 displays percentage of exhausted CD8 T cells, subsets are based on the expression of PD-1, PVRIG, and TIGIT. PVRIG is most highly correlated with exhausted CD8 T cells (Rows 1-4), the largest percentage of which is shared with PD-1 and TIGIT respectively. These results are supported by the TGI and survival of mice in their CT26 syngeneic model. Combining anti-mPD-L1 and anti-mPVRIG treatments significantly decreases tumor size and increases overall survival (Figure 6).

Pre-Clinical Studies of Novel PVRIG Blocking Antibody COM701

Data gathered from mouse models and patient samples warranted the development of a human blocking antibody for PVRIG. A new treatment model was also devised, which includes blockade of the three checkpoints discussed earlier (Figure 7). Preliminary testing demonstrates that COM701 increases CD4 T cell proliferation and CD8 T cell cytotoxicity; this cytotoxic activity was further enhanced by blocking TIGIT (Fig 8). Synergistic effects were noted in the tri-blockade of COM701, anti-PD-1, and anti-TIGIT. IFN γ production is modestly increased by single blockade, co-blockade strengthens this effect further, and tri-blockade results in over 300% increase from control (Fig 9).

After confirming COM701 efficacy on healthy cells, the Pardoll lab examined COM701's effects on tumor derived cells. Ex vivo experiments on TILs from cancers of the kidney, endometrium, and ovary were analyzed for signs of activation. TILs were treated with COM701, COM902 (anti-TIGIT), or Pembrolizumab (anti-PD-1). Single agent treatment moderately increases IFN γ levels; however, co-blockade with COM701 and COM902 most promote IFN γ secretion (Fig 10). A more comprehensive study of TIL activation was examined

in lung cancer. IFN γ , tumor necrosis factor alpha, and IL-2 displayed higher increases with co-blockade using COM701 and Pembrolizumab than controls or individual treatments (Fig 11).

Discussion

PD-1 therapy has become the standard of care for some malignancies; unfortunately, not all patients respond to this treatment, and not all responders achieve durable remission. Combination therapy is believed to be one method for advancing the success of checkpoint blockade (60). CTLA-4 has been used in conjunction with PD-1 and has improved patient outcomes; however, this progress has come at the cost of intensifying immune-related adverse effects (irAE) (61). These irAEs prevent patients from being able to continue therapy and can be lethal. Although the benefits outweigh the risks, there is a desire for additional options for combination checkpoint blockade.

Pardoll and colleagues provide another actionable target for checkpoint blockade. PVRIG is a novel inhibitory receptor. Co-blockade of PVRIG + TIGIT or PVRIG + PD-L1 inhibits tumor growth in B16gp100 and CT26 models respectively (Fig 3, 6). In vitro tri-blockade of PD-1, PVRIG, and TIGIT maximally enhances T cell IFN γ production. Blockade with COM701 alone and especially in combination with Pembrolizumab or COM902 blocking antibodies has also been shown to enhance activation of patient TILs from endometrial, ovarian, and lung cancers (Fig 10,11).

PVRL2 expression in tumors further enhances the viability of COM701 as a novel immunotherapy. PVRL2 maintains a similar expression profile in tumors regardless of PD-L1 status, meaning that COM701 can be indicated for a specific cancer even if the tumor is PD-L1⁻ (Fig 12). PVRL2 is induced by lung, ovarian, breast, and colon tumors (Fig 13). This is an

important aspect for combining with PD-1 blockade since the relatively low toxicity of anti-PD-1 therapy is believed to be due to its primarily tumor induced function (62). For this reason, COM701 co-blockade is expected to have less irAE than CTLA-4 co-blockade. The body of work produced by Pardoll and colleagues on PVRIG has successfully cleared COM701 for phase 1 clinical trials (63).

Next steps for COM701 will involve translating data from the bedside to the bench. The mechanisms behind clinical responses will need to be elucidated and a predictive algorithm for patient response will have to be designed. Identification of a soluble biomarker from blood samples would be ideal, as it can be used to predict and track patient responses. Tumor samples should be used to examine immune infiltrate and changes in protein expression throughout treatment. Finally, phenotyping of TILs will provide information on their activation status and therefore efficacy of therapy.

Figures

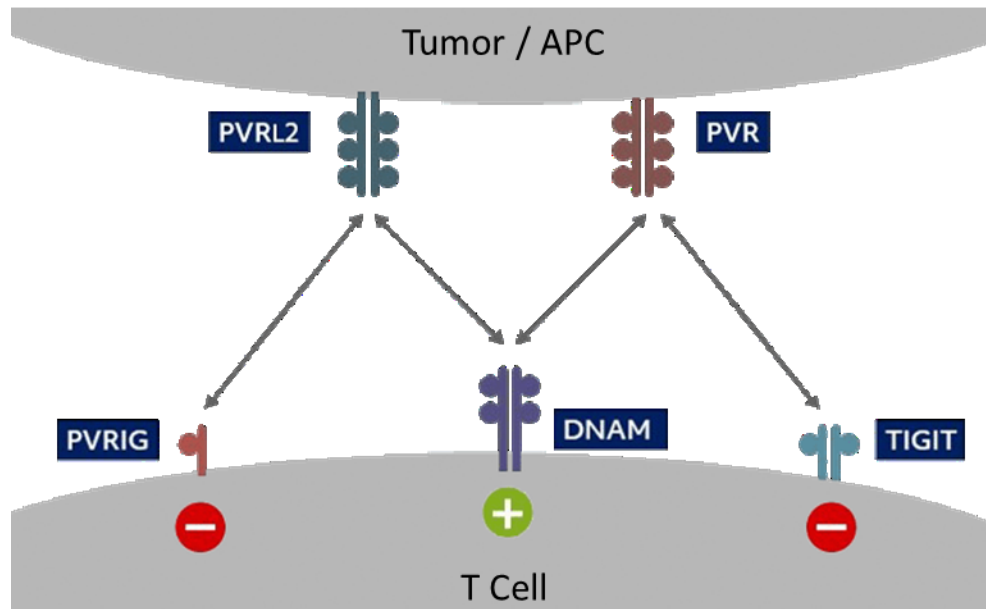


Figure 1: PVRIG is parallel, non-redundant immune checkpoint in the DNAM/TIGIT axis

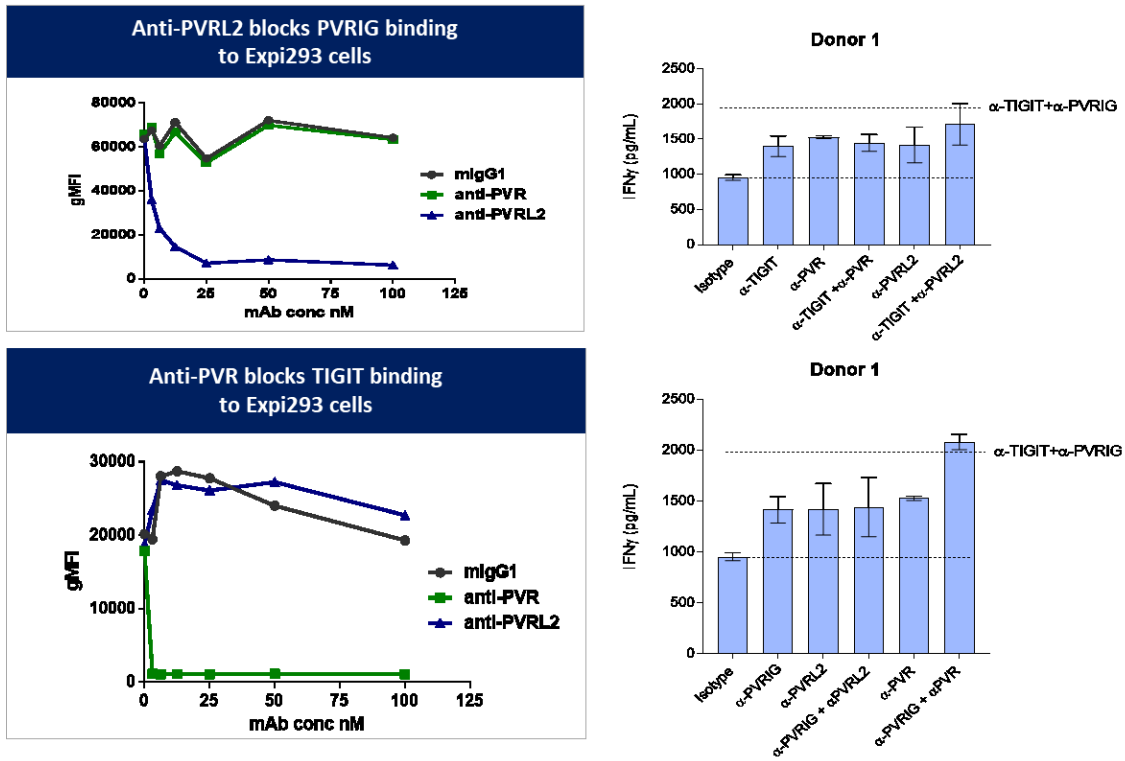


Figure 2: A-Left) Binding of PVRIG/TIGIT with increasing concentration of anti-PVR/PVRL2
Figure 2: B-Right) IFN γ secretion following blockade of PVR, PVRIG, PVRL2, and/or TIGIT

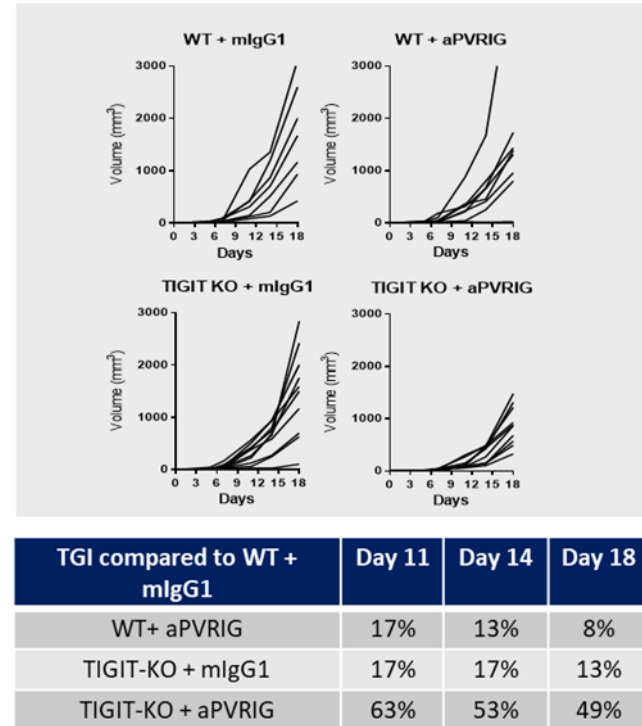
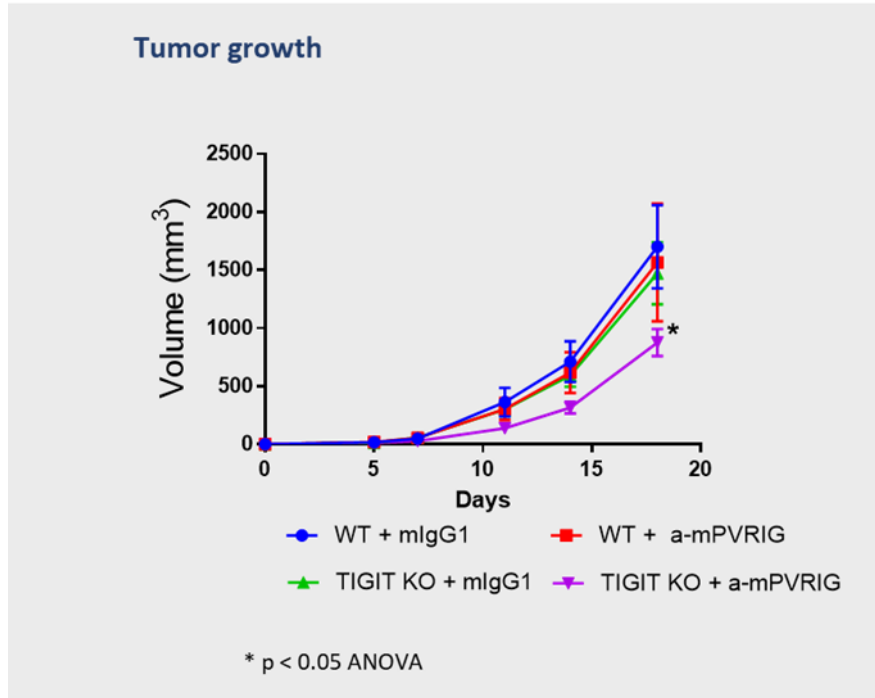


Figure 3: Tumor growth of WT or TIGIT KO mice with control or anti-mPVRIG

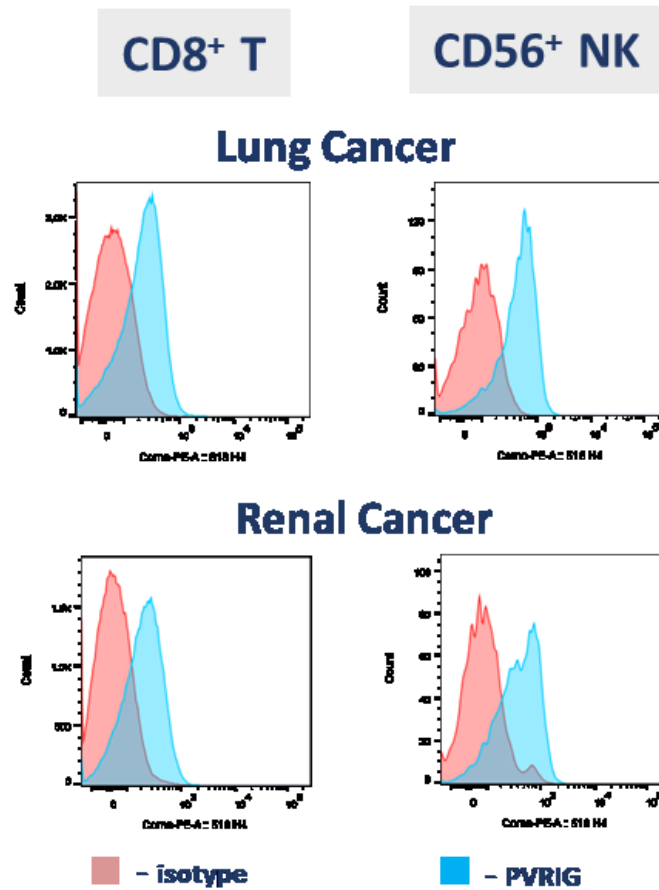


Figure 4: PVRIG binding on CD8 T cells and NK cells on TILs from lung cancer and renal cancer patients

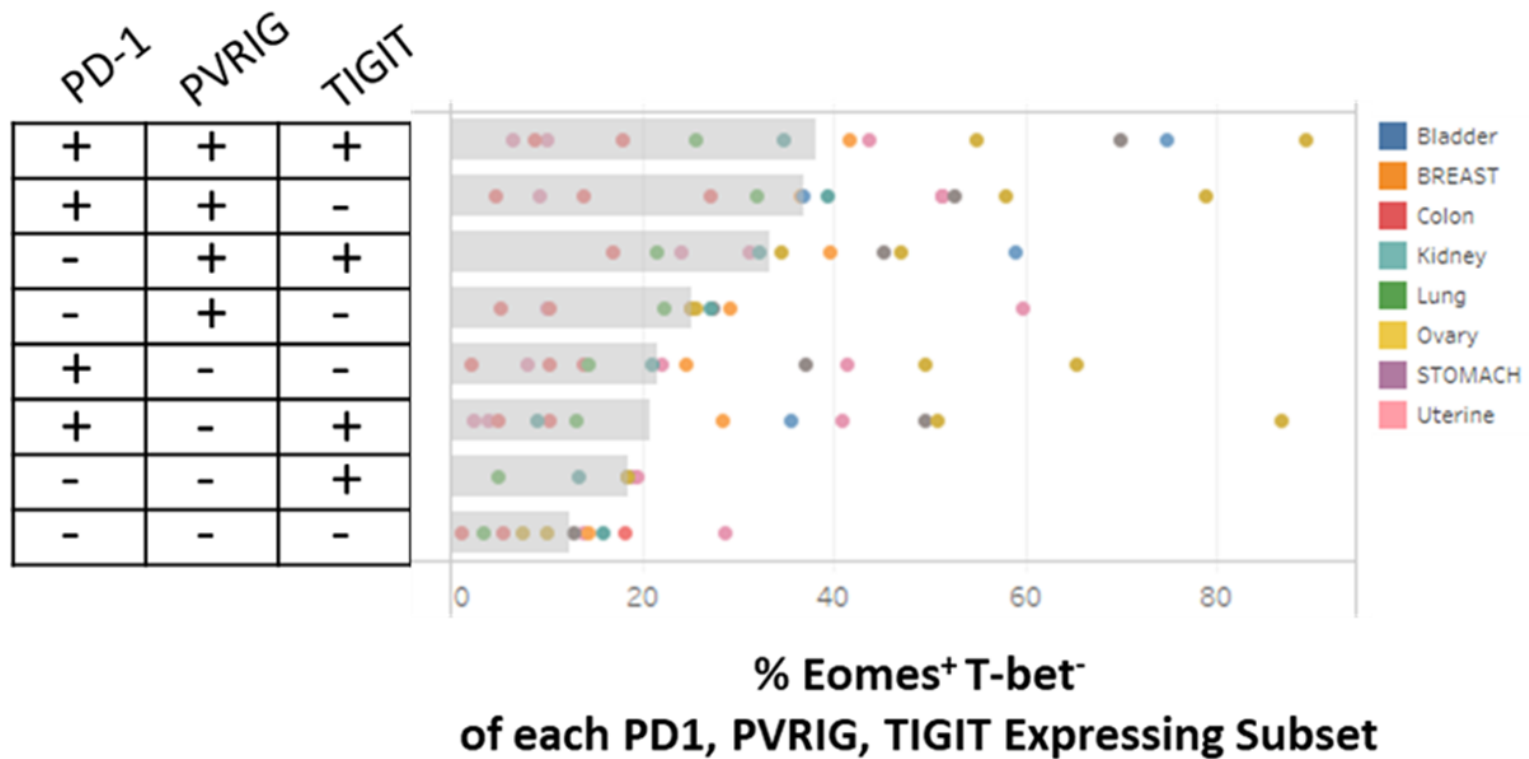


Figure 5: Expression of PD-1, PVRIG, and /or TIGIT on exhausted T cells from various cancers

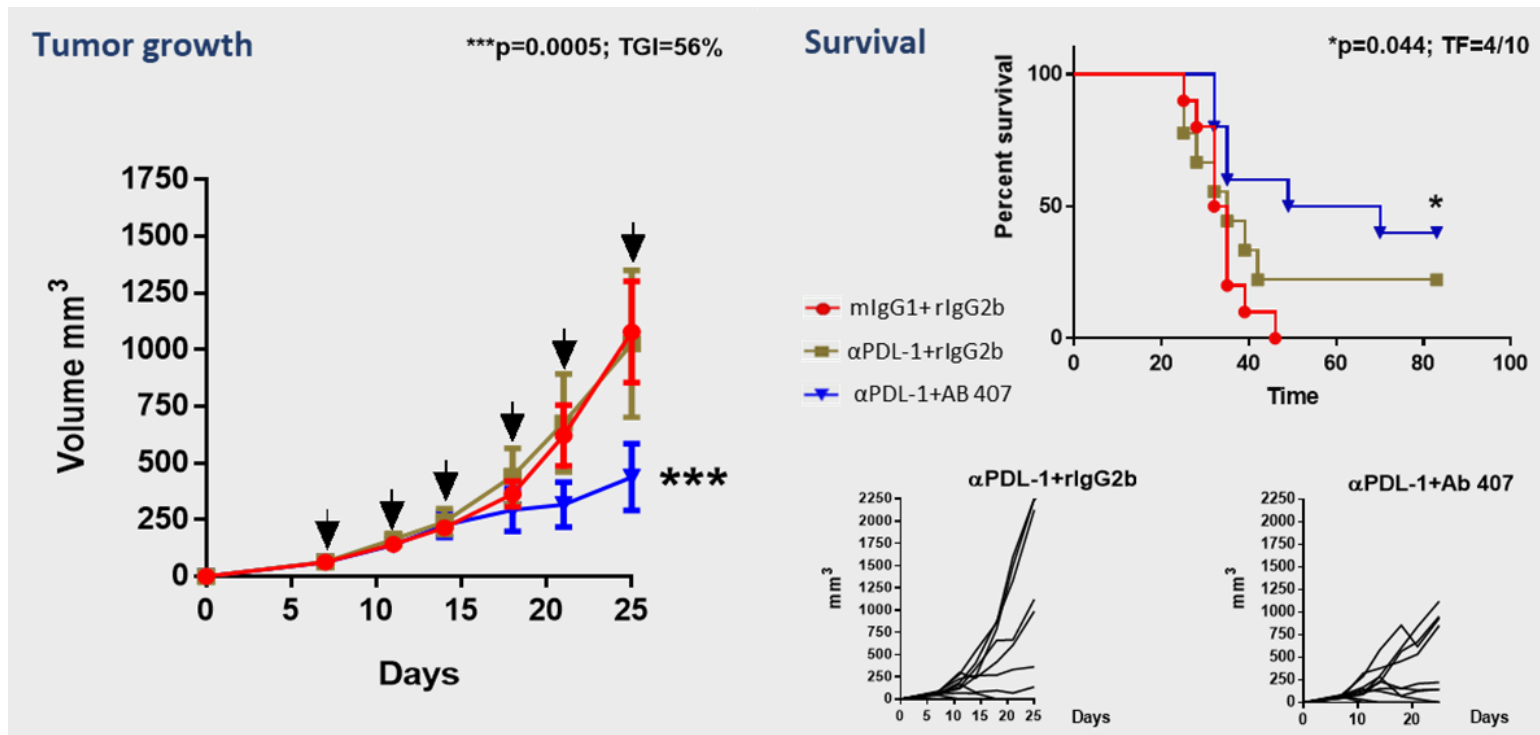


Figure 6: TGI and survival plots of mice treated with control, anti-mPD-L1, and/or anti-mPVRIG (Ab 407)

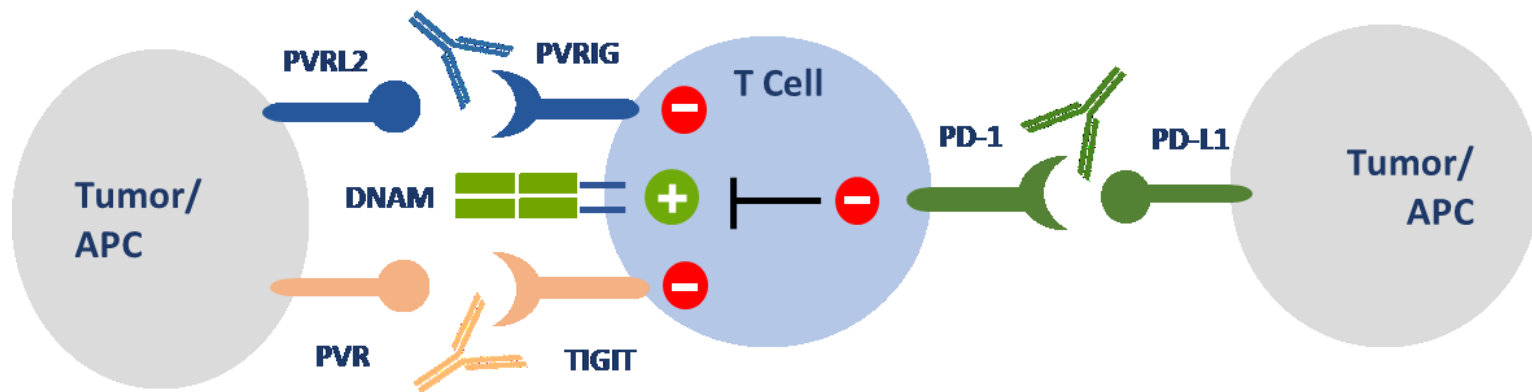
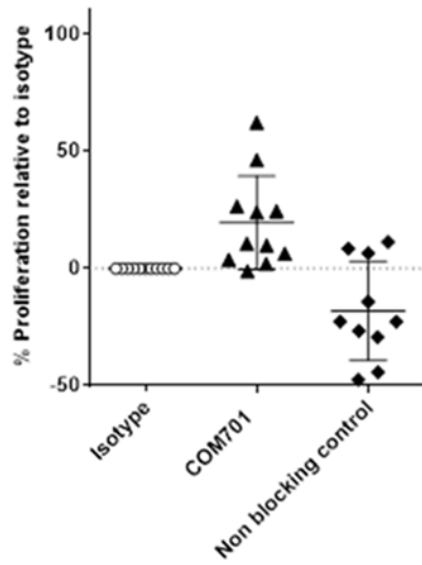


Figure 7: Rationale for combination therapy with anti-PD-1, anti-PVRIG, and anti-TIGIT.

Increased CD4⁺ T cell proliferation



Enhanced CD8⁺ cytotoxic activity

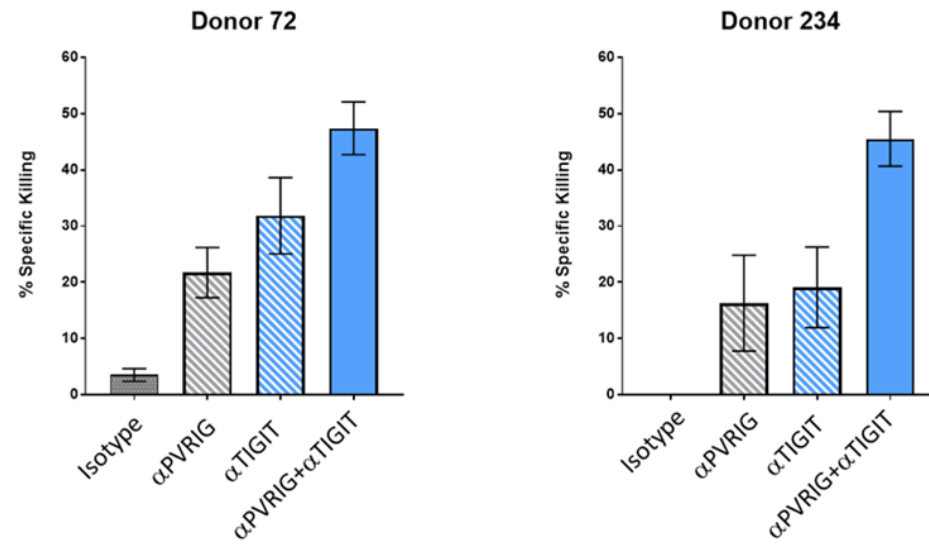


Figure 8: COM701 effects on CD4 T cell proliferation and CD8 T cell killing using healthy donor cells

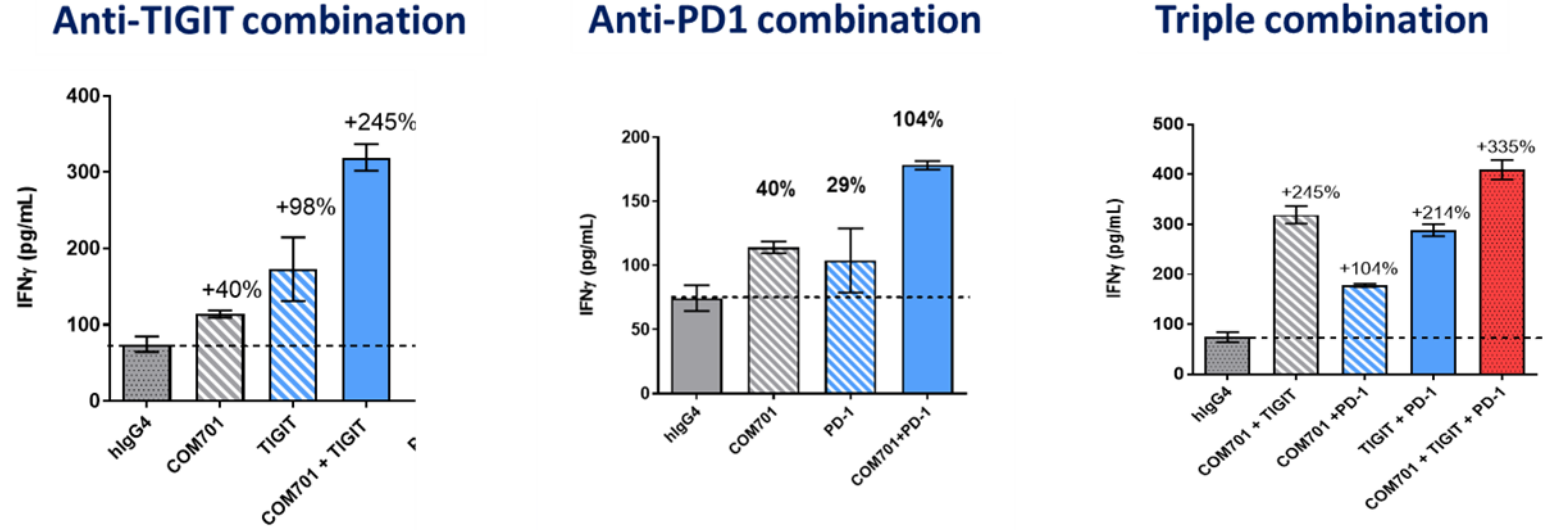


Figure 9: IFN γ production following combination blockade with COM701, anti-PD-1, and/or anti-TIGIT.

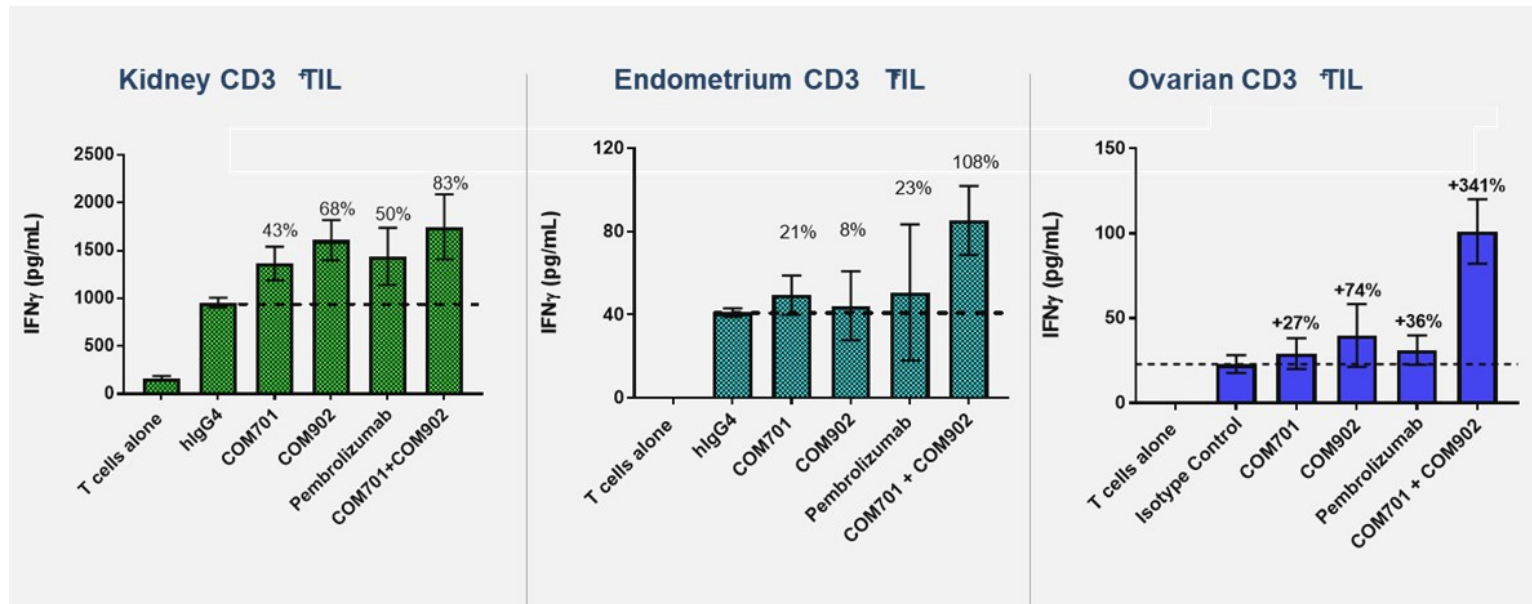


Figure 10: IFN γ secretion from TILs extracted from various cancers following blockade of Pembrolizumab (anti-PD-1) or COM701 and/or COM902 (anti-TIGIT)

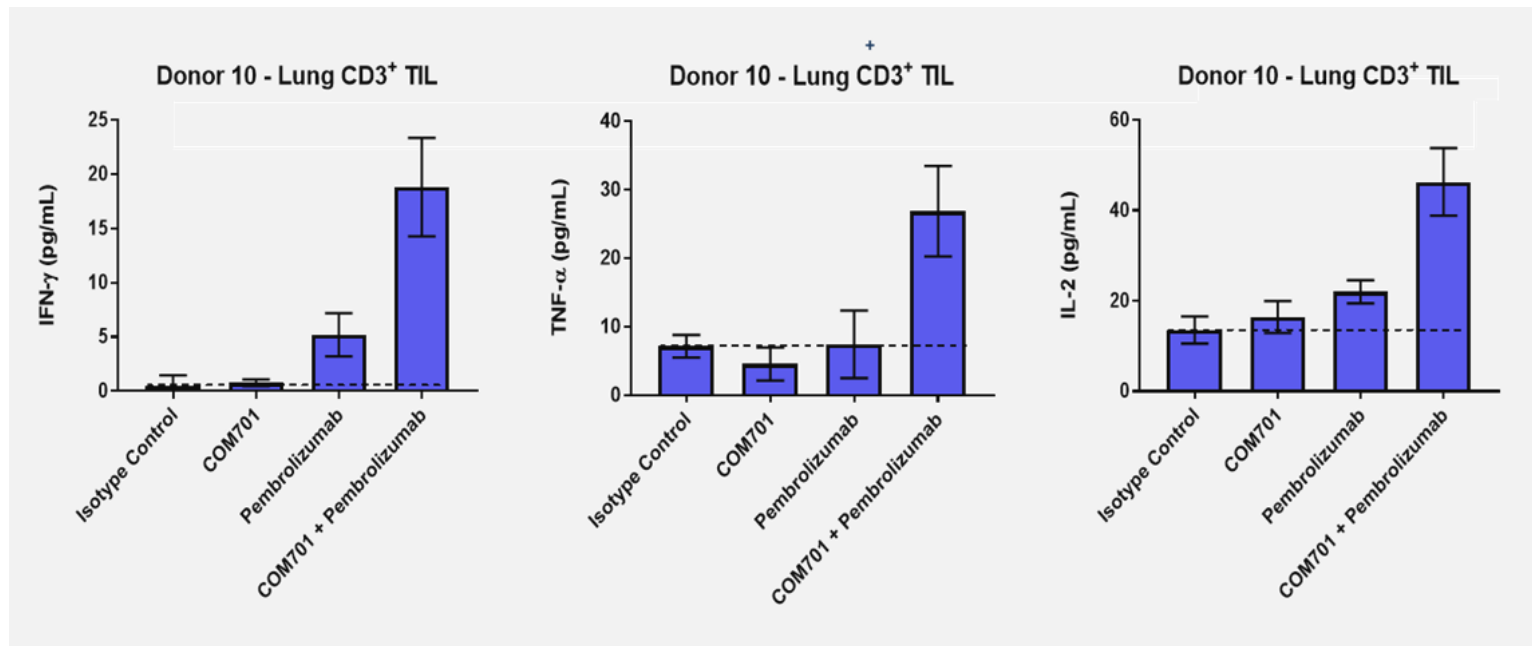


Figure 11: Lung CD3 TIL secretion of pro-inflammatory cytokines following blockade with COM701 and/or Pembrolizumab

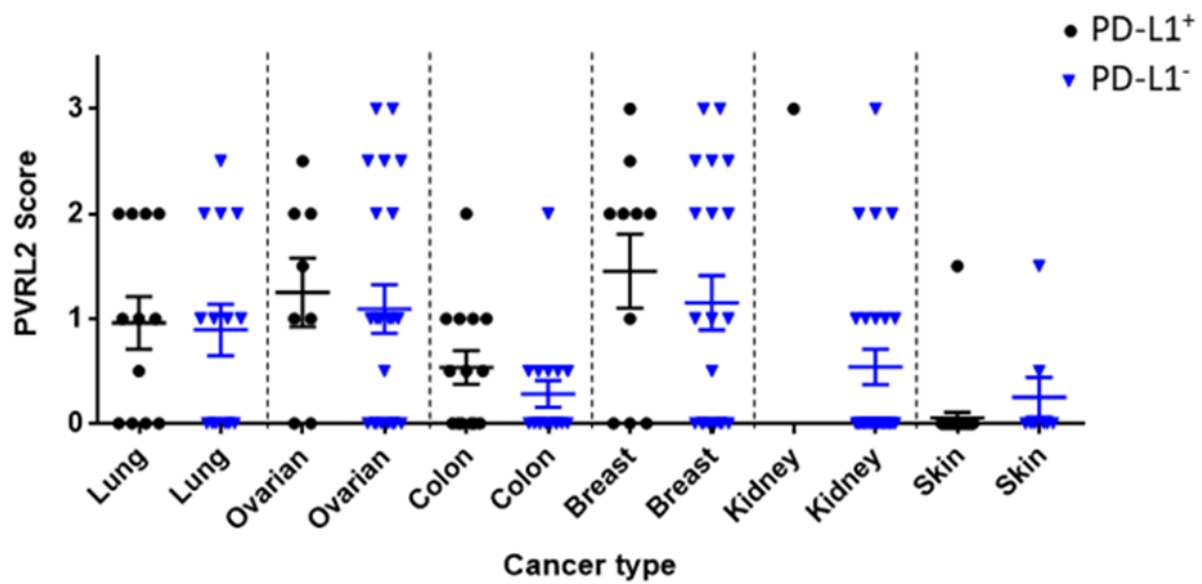


Figure 12: PVRL2 expression on PD-L1⁺ or PD-L1⁻ tumors

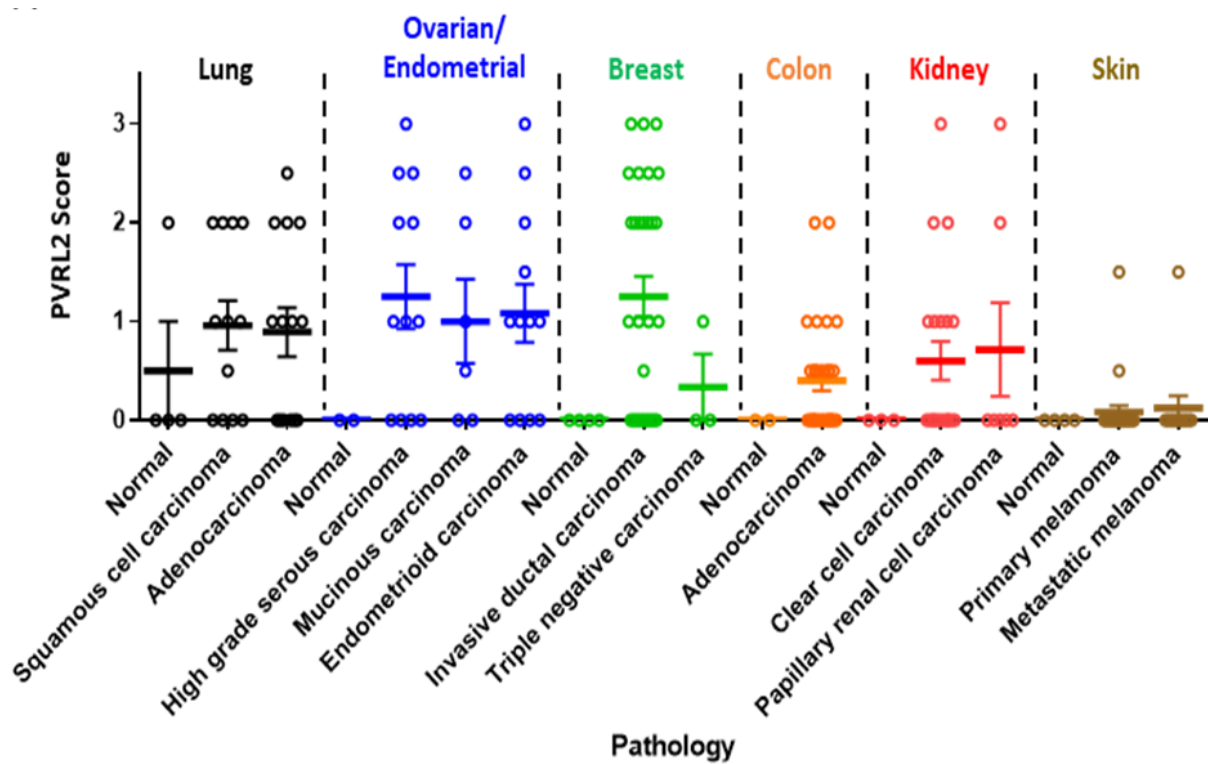


Figure 13: PVRL2 expression on various normal and malignant tissues.

Works Cited

1. Siegel R, Miller K, Jemal A. Cancer statistics, 2018. *CA Cancer J Clin.* 2018;68(1):7-30. doi:10.3322/caac.21442
2. Stewart B. World Cancer Report 2014. Lyon: International Agency for Research in Cancer; 2014.
3. Evolution of Cancer Treatments: Chemotherapy | American Cancer Society. Cancer.org. <https://www.cancer.org/cancer/cancer-basics/history-of-cancer/cancer-treatment-chemo.html>. Published 2014. Accessed July 25, 2018.
4. Ikushima H. Radiation therapy: state of the art and the future. *The Journal of Medical Investigation.* 2010;57(1,2):1-11. doi:10.2152/jmi.57.1
5. Monoclonal antibodies to treat cancer | American Cancer Society. Cancer.org. <https://www.cancer.org/treatment/treatments-and-side-effects/treatment-types/immunotherapy/monoclonal-antibodies.html>. Published 2016. Accessed July 25, 2018.
6. Immune checkpoint inhibitors to treat cancer. Cancer.org. <https://www.cancer.org/treatment/treatments-and-side-effects/treatment-types/immunotherapy/immune-checkpoint-inhibitors.html>. Published 2017. Accessed July 25, 2018.
7. Grosso JF, Jure-kunkel MN. CTLA-4 blockade in tumor models: an overview of preclinical and translational research. *Cancer Immun.* 2013;13:5.
8. Xu F, Sunderland A, Zhou Y, Schulick R, Edil B, Zhu Y. Blockade of CD112R and TIGIT signaling sensitizes human natural killer cell functions. *Cancer Immunology, Immunotherapy.* 2017;66(10):1367-1375. doi:10.1007/s00262-017-2031-x
9. DeVita V, Chu E. A History of Cancer Chemotherapy. *Cancer Res.* 2008;68(21):8643-8653. doi:10.1158/0008-5472.can-07-6611
10. Cancer in the Sixteenth to Eighteenth Centuries | American Cancer Society. Cancer.org. <https://www.cancer.org/cancer/cancer-basics/history-of-cancer/sixteenth-to-eighteenth-centuries.html>. Published 2014. Accessed July 27, 2018.
11. Cancer in the Nineteenth Century | American Cancer Society. Cancer.org. <https://www.cancer.org/cancer/cancer-basics/history-of-cancer/nineteenth-century.html>. Published 2014. Accessed July 27, 2018.

12. Development of Modern Knowledge about Cancer Causes | American Cancer Society. Cancer.org. <https://www.cancer.org/cancer/cancer-basics/history-of-cancer/modern-knowledge-and-cancer-causes.html>. Published 2014. Accessed July 27, 2018.
13. How Is Acute Myeloid Leukemia Classified?. Cancer.org. <https://www.cancer.org/cancer/acute-myeloid-leukemia/detection-diagnosis-staging/how-classified.html>. Published 2016. Accessed July 27, 2018.
14. Hortobagyi G. *Physician To Physician AJCC 8Th Edition Breast*. American Joint Committee on Cancer; 2017:18-50. https://cancerstaging.org/CSE/Physician/Documents/AJCC_PPT%20-Breast%20Webinar%2011-8-17.pdf. Accessed July 27, 2018.
15. Baskar R, Lee K, Yeo R, Yeoh K. Cancer and Radiation Therapy: Current Advances and Future Directions. *Int J Med Sci*. 2012;9(3):193-199. doi:10.7150/ijms.3635
16. Gerber DE, Chan TA. Recent advances in radiation therapy. *Am Fam Physician*. 2008;78(11):1254-62.
17. Brenner DJ, Hall EJ. The origins and basis of the linear-quadratic model. *Int J Radiat Oncol Biol Phys*. 1992;23(1):252-3.
18. Rembielak A, Woo TC. Intensity-modulated radiation therapy for the treatment of pediatric cancer patients. *Nat Clin Pract Oncol*. 2005;2(4):211-7.
19. Stea B, Hazard LJ, Gonzalez VJ, Hamilton R. The role of radiation therapy in the control of locoregional and metastatic cancer. *Int J Surg Oncol*. 2011;103(6):627-38.
20. Harper D. chemotherapy | Origin and meaning of chemotherapy by Online Etymology Dictionary. Etymonline.com. <https://www.etymonline.com/word/chemotherapy>. Accessed July 28, 2018.
21. Definition of CHEMOTHERAPY. Merriam-webster.com. <https://www.merriam-webster.com/dictionary/chemotherapy>. Accessed July 28, 2018.
22. Gilman A. Therapeutic applications of chemical warfare agents. *Fed Proc*. 1946;5:285-92.
23. Goodman LS, Wintrobe MM. Nitrogen mustard therapy; use of methyl-bis (beta-chloroethyl) amine hydrochloride and tris (beta-chloroethyl) amine hydrochloride for Hodgkin's disease, lymphosarcoma, leukemia and certain allied and miscellaneous disorders. *J Am Med Assoc*. 1946;132:126-32.
24. Farber S, Diamond LK. Temporary remissions in acute leukemia in children produced by folic acid antagonist, 4-aminopteroyl-glutamic acid. *N Engl J Med*. 1948;238(23):787-93.
25. Elion GB, Singer S, Hitchings GH. Antagonists of nucleic acid derivatives. VIII. Synergism in combinations of biochemically related antimetabolites. *J Biol Chem*. 1954;208(2):477-88.

26. Druker BJ, Tamura S, Buchdunger E, et al. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. *Nat Med*. 1996;2(5):561-6.
27. Baran Y, Saydam G. Cumulative clinical experience from a decade of use: imatinib as first-line treatment of chronic myeloid leukemia. *J Blood Med*. 2012;3:139-50.
28. Miller KD, Siegel RL, Lin CC, et al. Cancer treatment and survivorship statistics, 2016. *CA Cancer J Clin*. 2016;66(4):271-89.
29. Littman DR. Releasing the Brakes on Cancer Immunotherapy. *Cell*. 2015;162(6):1186-90.
30. Pardoll DM, Topalian SL. The role of CD4+ T cell responses in antitumor immunity. *Curr Opin Immunol*. 1998;10(5):588-94.
31. Pardoll DM. Inducing autoimmune disease to treat cancer. *Proc Natl Acad Sci USA*. 1999;96(10):5340-2.
32. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer*. 2012;12(4):252-64.
33. Collin M. Immune checkpoint inhibitors: a patent review (2010-2015). *Expert Opin Ther Pat*. 2016;26(5):555-64.
34. Okazaki T, Honjo T. PD-1 and PD-1 ligands: from discovery to clinical application. *Int Immunol*. 2007;19(7):813-24.
35. Dong H, Zhu G, Tamada K, Chen L. B7-H1, a third member of the B7 family, co-stimulates T-cell proliferation and interleukin-10 secretion. *Nat Med*. 1999;5(12):1365-9.
36. Tseng SY, Otsuji M, Gorski K, et al. B7-DC, a new dendritic cell molecule with potent costimulatory properties for T cells. *J Exp Med*. 2001;193(7):839-46.
37. Freeman GJ, Long AJ, Iwai Y, et al. Engagement of the PD-1 immunoinhibitory receptor by a novel B7 family member leads to negative regulation of lymphocyte activation. *J Exp Med*. 2000;192(7):1027-34.
38. Pardoll DM. Immunology beats cancer: a blueprint for successful translation. *Nat Immunol*. 2012;13(12):1129-32.
39. Latchman Y, Wood CR, Chernova T, et al. PD-L2 is a second ligand for PD-1 and inhibits T cell activation. *Nat Immunol*. 2001;2(3):261-8.
40. Bardhan K, Anagnostou T, Boussiotis VA. The PD1:PD-L1/2 Pathway from Discovery to Clinical Implementation. *Front Immunol*. 2016;7:550.

41. Brown JA, Dorfman DM, Ma FR, et al. Blockade of programmed death-1 ligands on dendritic cells enhances T cell activation and cytokine production. *J Immunol.* 2003;170(3):1257-66.
42. Pentcheva-hoang T, Chen L, Pardoll DM, Allison JP. Programmed death-1 concentration at the immunological synapse is determined by ligand affinity and availability. *Proc Natl Acad Sci USA.* 2007;104(45):17765-70.
43. Brahmer JR, Drake CG, Wollner I, et al. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. *J Clin Oncol.* 2010;28(19):3167-75.
44. Lipson EJ, Sharfman WH, Drake CG, et al. Durable cancer regression off-treatment and effective reinduction therapy with an anti-PD-1 antibody. *Clin Cancer Res.* 2013;19(2):462-8.
45. Yu X, Harden K, Gonzalez LC, et al. The surface protein TIGIT suppresses T cell activation by promoting the generation of mature immunoregulatory dendritic cells. *Nat Immunol.* 2009;10(1):48-57.
46. Stanietsky N, Simic H, Arapovic J, et al. The interaction of TIGIT with PVR and PVRL2 inhibits human NK cell cytotoxicity. *Proc Natl Acad Sci USA.* 2009;106(42):17858-63.
47. Boles KS, Vermi W, Facchetti F, et al. A novel molecular interaction for the adhesion of follicular CD4 T cells to follicular DC. *Eur J Immunol.* 2009;39(3):695-703.
48. Joller N, Hafler JP, Brynedal B, et al. Cutting edge: TIGIT has T cell-intrinsic inhibitory functions. *J Immunol.* 2011;186(3):1338-42.
49. Lozano E, Dominguez-villar M, Kuchroo V, Hafler DA. The TIGIT/CD226 axis regulates human T cell function. *J Immunol.* 2012;188(8):3869-75.
50. Johnston RJ, Comps-agrar L, Hackney J, et al. The immunoreceptor TIGIT regulates antitumor and antiviral CD8(+) T cell effector function. *Cancer Cell.* 2014;26(6):923-937.
51. Liu S, Zhang H, Li M, et al. Recruitment of Grb2 and SHIP1 by the ITT-like motif of TIGIT suppresses granule polarization and cytotoxicity of NK cells. *Cell Death Differ.* 2013;20(3):456-64.
52. Zhang Q, Bi J, Zheng X, et al. Blockade of the checkpoint receptor TIGIT prevents NK cell exhaustion and elicits potent anti-tumor immunity. *Nat Immunol.* 2018;
53. Manieri NA, Chiang EY, Grogan JL. TIGIT: A Key Inhibitor of the Cancer Immunity Cycle. *Trends Immunol.* 2017;38(1):20-28.

54. Stamm H, Klingler F, Grossjohann EM, et al. Immune checkpoints PVR and PVRL2 are prognostic markers in AML and their blockade represents a new therapeutic option. *Oncogene*. 2018;
55. Wilson MD, Cheung J, Martindale DW, Scherer SW, Koop BF. Comparative analysis of the paired immunoglobulin-like receptor (PILR) locus in six mammalian genomes: duplication, conversion, and the birth of new genes. *Physiol Genomics*. 2006;27(3):201-18.
56. Zhu Y, Panicia A, Schulick AC, et al. Identification of CD112R as a novel checkpoint for human T cells. *J Exp Med*. 2016;213(2):167-76.
57. Lee NH, Kim M, Oh SY, Kim SG, Kwon HC, Hwang TH. Gene expression profiling of hematologic malignant cell lines resistant to oncolytic virus treatment. *Oncotarget*. 2017;8(1):1213-1225.
58. Xu F, Sunderland A, Zhou Y, Schulick RD, Edil BH, Zhu Y. Blockade of CD112R and TIGIT signaling sensitizes human natural killer cell functions. *Cancer Immunol Immunother*. 2017;66(10):1367-1375.
59. Dong Y, Sun Q, Zhang X. PD-1 and its ligands are important immune checkpoints in cancer. *Oncotarget*. 2017;8(2):2171-2186.
60. Kyi C, Postow MA. Immune checkpoint inhibitor combinations in solid tumors: opportunities and challenges. *Immunotherapy*. 2016;8(7):821-37.
61. Michot JM, Bigenwald C, Champiat S, et al. Immune-related adverse events with immune checkpoint blockade: a comprehensive review. *Eur J Cancer*. 2016;54:139-48.
62. Pennock GK, Chow LQ. The Evolving Role of Immune Checkpoint Inhibitors in Cancer Treatment. *Oncologist*. 2015;20(7):812-22.
63. Elana Holzman. *Compugen Announces FDA Clearance Of IND Application For COM701, A First-In-Class Immuno-Oncology Therapeutic Antibody.*; 2018. <https://www.cgen.com/wp-content/uploads/COM701-IND-clearance-Final-02-07-2018.pdf>. Accessed July 30, 2018.

CURRICULUM VITAE
The Johns Hopkins University School of Medicine

Matthew Neil

January 3rd, 1987

Educational History:

M.S. expected	2018	Graduate Program in Immunology Mentor: Drew Pardoll, MD-PhD	Johns Hopkins School of Medicine
M.S.	2014	Biology Mentor: Sherine Elsawa, PhD	Northern Illinois University
B.S.	2011	Biology	University of Illinois - Chicago
H.S.	2005		Aurora Christian School

Publications:

Boin F, Giardino Torchia ML, Borrello I, Noonan KA, **Neil M**, Soloski MJ, Cimbri R. Flow cytometric discrimination of seven lineage markers by using two fluorochromes. PLoS ONE. 2017;12(11):e0188916.

Jackson DA, Smith TD, Amarsaikhan N, Han W, **Neil MS**, Boi SK, Vrabel AM, Tolosa EJ, Almada LL, Fernandez-Zapico ME, Elsawa SF. Modulation of the IL-6 Receptor α Underlies GLI2-Mediated Regulation of Ig Secretion in Waldenström Macroglobulinemia Cells. J Immunol. 2015;195(6):2908-16.

Han W, Jackson DA, Matissek SJ, Misurelli JA, **Neil MS**, Sklavanitis B, Amarsaikhan N, Elsawa SF. Novel Molecular Mechanism of Regulation of CD40 Ligand by the Transcription Factor GLI2. J Immunol. 2017;198(11):4481-4489.

Teaching and Tutoring Experience:

03/2017-09/2017	Tutored students ranging from grade school to community college
08/2013-05/2014	Taught an introductory biology lab at Northern Illinois University
08/2009-05/2011	Worked as a peer mentor for residence halls at University of Illinois Chicago
01/2004-01/2005	Hired as a private tutor for high school classmate
08/2004-05-2005	Enrolled in a peer tutor class for middle school, freshman, and sophomore students